

## CLAIMS

1. A method of determining candidate functional mediators of a selected gene, comprising the steps of:
  - a. altering expression of a first selected gene in a first of two  
5 populations of identical cells;
  - b. comparing expression levels of a set of genes in the two populations of cells;
  - c. identifying genes in the set whose expression levels differ  
10 between the two populations of cells, wherein the genes identified are candidate functional mediators of the selected gene;
  - d. altering expression of a second selected gene in one of a third and fourth populations of cells, wherein the third and fourth populations comprise identical cells, wherein the second selected gene is a candidate functional mediator of the first selected gene;
  - 15 e. comparing expression levels of a set of genes in the third and fourth populations of cells;
  - f. identifying genes in the set whose expression levels differ between the third and fourth populations of cells, wherein the genes identified are candidate functional mediators of the second selected gene.
- 20 2. The method of claim 1 wherein the step of altering (a) comprises adding an exogenous regulatory agent to the first population of cells, wherein the regulatory molecule alters expression of the first selected gene.
- 25 3. The method of claim 1 wherein the step of altering (d) comprises adding an exogenous regulatory agent to the third populations of cells, wherein the regulatory molecule alters expression of the second selected gene.
4. The method of claim 1 wherein the step of altering (a) comprises mutagenizing the first selected gene.

5. The method of claim 1 wherein the step of altering (d) comprises mutagenizing the second selected gene.
6. The method of claim 1 wherein the step of altering (a) comprises administering an antisense construct to the first population, wherein  
5 the antisense construct encodes an RNA which is complementary to mRNA transcribed from the first selected gene.
7. The method of claim 1 wherein the step of altering (d) comprises administering an antisense construct to the third population, wherein  
10 the antisense construct encodes an RNA which is complementary to mRNA transcribed from the second selected gene.
8. The method of claim 1 wherein the step of altering (a) comprises administering a ribozyme construct to the first population, wherein  
the ribozyme construct encodes an RNA which cleaves mRNA transcribed from the first selected gene.
9. The method of claim 1 wherein the step of altering (d) comprises administering a ribozyme construct to the third population, wherein  
15 the ribozyme construct encodes an RNA which cleaves mRNA transcribed from the second selected gene.
10. The method of claim 1 wherein the step of altering (a) comprises  
20 altering copy number of the first selected gene in the first population of cells.
11. The method of claim 1 wherein the step of altering (d) comprises altering copy number of the second selected gene in the third population of cells.
12. The method of claim 1 wherein steps (d-f) are repeated with a  
25 plurality of candidate functional mediators identified in step (c).
13. The method of claim 1 wherein steps (a-c) are repeated using a candidate functional mediator of the second selected gene as a first selected gene.

14. The method of claim 1 wherein steps (a-f) are repeated using a candidate functional mediator of the second selected gene as a first selected gene.
- 5 15. The method of claim 1 wherein the first and second selected genes are tumor suppressor genes.
16. The method of claim 1 wherein the first and second selected genes are oncogenes.
17. A method of identifying pathways of functional mediators of a selected gene, comprising the steps of:
- 10 a. altering expression of a first selected gene in a first of two populations of identical cells;
- b. comparing expression levels of a set of genes in the two populations of cells;
- 15 c. identifying genes in the set whose expression levels differ between the two populations of cells, wherein the genes identified form a set of candidate functional mediators of the first selected gene;
- d. altering expression of a second selected gene in one of a third and fourth populations of cells, wherein the third and fourth populations comprise identical cells;
- 20 e. comparing expression levels of the set of genes in the third and fourth populations of cells;
- f. identifying genes in the set whose expression levels differ between the third and fourth populations of cells, wherein the genes identified form a set of candidate functional mediators of the second selected gene;
- 25 g. comparing the candidate functional mediators identified of the first and second selected genes, wherein genes which are identified as candidate functional mediators of both selected genes suggest that the first and second selected genes are components of a common pathway, wherein failure to identify a candidate functional mediator of both selected genes suggests that the two selected genes are in different pathways, wherein
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identification of the set of candidate functional mediators of the first selected gene as a subset of the set of candidate functional mediators of the second selected gene suggests that the first selected gene is downstream in a pathway relative to the second selected gene, and wherein a candidate functional mediator which is identified in only one of the two sets of candidate functional mediators is identified as upstream in the pathway of a selected gene from a point of convergence with the pathway of the other selected gene, if the sets of candidate functional mediator genes of the first and second selected genes contain common members.

- 10 18. The method of claim 17 wherein the step of altering (a) comprises adding an exogenous regulatory agent to the first population of cells, wherein the regulatory molecule alters expression of the first selected gene.
- 15 19. The method of claim 17 wherein the step of altering (d) comprises adding an exogenous regulatory agent to the third populations of cells, wherein the regulatory molecule alters expression of the second selected gene.
- 20 20. The method of claim 17 wherein the step of altering (a) comprises mutagenizing the first selected gene.
- 20 21. The method of claim 17 wherein the step of altering (d) comprises mutagenizing the second selected gene.
- 25 22. The method of claim 17 wherein the step of altering (a) comprises administering an antisense construct to the first population, wherein the antisense construct encodes an RNA which is complementary to mRNA transcribed from the first selected gene.
23. The method of claim 17 wherein the step of altering (d) comprises administering an antisense construct to the third population, wherein the antisense construct encodes an RNA which is complementary to mRNA transcribed from the second selected gene.
- 30 24. The method of claim 17 wherein the step of altering (a) comprises administering a ribozyme construct to the first population, wherein

the ribozyme construct encodes an RNA which cleaves mRNA transcribed from the first selected gene.

25. The method of claim 17 wherein the step of altering (d) comprises administering a ribozyme construct to the third population, wherein the ribozyme construct encodes an RNA which cleaves mRNA transcribed from the second selected gene.

26. The method of claim 17 wherein the step of altering (a) comprises altering copy number of the first selected gene in the first population of cells.

27. The method of claim 17 wherein the step of altering (d) comprises altering copy number of the second selected gene in the third population of cells.

28. The method of claim 17 wherein the first and second selected genes are tumor suppressor genes.

29. The method of claim 17 wherein the first and second selected genes are oncogenes.

30. A method to determine a pathway of gene products, comprising the step of:

comparing a first set of candidate functional mediator genes identified by the process of:

(a) comparing expression levels of a set of genes in two populations of identical cells, wherein a first of the two populations of cells has been treated to alter expression of a first selected gene,

(b) identifying genes in the set whose expression levels differ between the two populations of cells, wherein the genes identified are candidate functional mediators of the first selected gene;

with a second set of candidate functional mediator genes identified by the process of:

5 (c) comparing expression levels of the set of genes in a third and fourth population of cells, wherein the third population of cells has been treated to alter expression of a second selected gene;

10 (d) identifying genes whose expression levels differ between the third and fourth populations of identical cells, wherein the genes identified are candidate functional mediators of the second selected gene;

identifying the first and second selected genes as components of a common pathway when one or more genes are found to be candidate functional mediators of both of said first and said second selected genes.

15 31. A method to determine a pathway of gene products, comprising the step of:

comparing a first set of candidate functional mediator genes identified by the process of:

20 (a) comparing expression levels of a set of genes in two populations of identical cells, wherein a first of the two populations of cells has been treated to alter expression of a first selected gene,

25 (b) identifying genes in the set whose expression levels differ between the two populations of cells, wherein the genes identified are candidate functional mediators of the first selected gene;



with a second set of candidate functional mediator genes identified by the process of:

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(c) comparing expression levels of the set of genes in a third and fourth population of cells, wherein the third population of cells has been treated to alter expression of a second selected gene;

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(d) identifying genes whose expression levels differ between the third and fourth populations of identical cells, wherein the genes identified are candidate functional mediators of the second selected gene;

identifying the first and second selected genes as being in different pathways when no gene is identified as being a candidate functional mediator of both of said first and said second selected genes.

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32. A method to determine a pathway of gene products, comprising the step of:

comparing a first set of candidate functional mediator genes identified by the process of:

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(a) comparing expression levels of a set of genes in two populations of identical cells, wherein a first of the two populations of cells has been treated to alter expression of a first selected gene;

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(b) identifying genes in the set whose expression levels differ between the two populations of cells, wherein the genes identified are candidate functional mediators of the first selected gene;

with a second set of candidate functional mediator genes identified by the process of:

- 5 (c) comparing expression levels of the set of genes in a third and fourth population of identical cells, wherein the third population of cells has been treated to alter expression of a second selected gene;
- 10 (d) identifying genes whose expression levels differ between the third and fourth populations of cells, wherein the genes identified are candidate functional mediators of the second selected gene;

15 identifying a gene which is identified as a candidate functional mediator of only one of said first and said second selected genes as upstream in the pathway of the first or second selected gene from a point of convergence with the pathway of the second or first selected gene, if the first and second sets of candidate functional mediator genes contain common members.

33. A method to determine a pathway of gene products, comprising the step of:

20 comparing a first set of candidate functional mediator genes identified by the process of:

- 25 (a) comparing expression levels of a set of genes in two populations of identical cells, wherein a first of the two populations of cells has been treated to alter expression of a first selected gene;
- (b) identifying genes in the set whose expression levels differ between the two populations of cells,



wherein the genes identified are candidate functional mediators of the first selected gene;

with a second set of candidate functional mediator genes identified by the process of:

- 5 (c) comparing expression levels of the set of genes in a third and fourth population of identical cells, wherein the third population of cells has been treated to alter expression of a second selected gene;
- 10 (d) identifying genes whose expression levels differ between the third and fourth populations of cells, wherein the genes identified are candidate functional mediators of the second selected gene;

15 identifying the first selected gene as downstream in a pathway relative to the second selected gene if the set of candidate functional mediators of the first selected gene is a subset of the set of candidate functional mediators of the second selected gene.